Sexual Behavior, Human Papillomavirus Type 16 (HPV 16) Infection, and HPV 16 Seropositivity

PHILIP E. CASTLE, PHD, MPH,* TAMMY SHIELDS, MPH,* REINHARD KIRNBAUER, MD,†
M. MICHELE MANOS, PHD, MPH,‡ ROBERT D. BURK, MD,§ ANDREW G. GLASS, MD,‡ DAVID R. SCOTT, MD,‖
MARK E. SHERMAN, MD,* AND MARK SCHIFFMAN, MD, MPH*

*From the *Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; †Division of Immunology, Allergy, and Infectious Diseases, Department of Dermatology, University of Vienna Medical School, Vienna, Austria; ‡Division of Research, The Permanente Medical Group, Oakland, California; §Albert Einstein College of Medicine, Bronx, New York; and ‖Kaiser Permanente Center for Health Research, Portland, Oregon*

Background: **Sexual behaviors have been linked to seropositivity for human papillomavirus (HPV) but not with the magnitude of the seroreactivity.**

Goals: **The objective of this analysis was to examine the association of sexual behavior, cervical HPV 16 DNA positivity at enrollment (past) and at diagnosis (current), and other potential determinants with the likelihood and magnitude of HPV 16 seropositivity at diagnosis.**

Study Design: **With use of stored specimens from an incidence case–control study at Kaiser Permanente (Portland, OR), women were tested for seroreactivity to HPV 16 by enzyme-linked immunosorbent assay with virus-like particles at diagnosis and were tested for past and concurrent cervical HPV 16 DNA positivity with MY09/MY11 L1 consensus primer PCR. Questionnaire data were used to ascertain past sexual behavior.**

Results: **Increased lifetime number of sex partners ($P_{Trend} < 0.001$), past HPV 16 DNA positivity (odds ratio = 6.9; 95% confidence interval = 1.5–31), and a current cytologic diagnosis ($P_{Trend} < 0.03$) were independently associated with HPV 16 seropositivity. Among the seropositive, only lifetime number of sex partners ($P_{Trend} < 0.001$) and past HPV 16 DNA positivity ($P = 0.003$) were independently associated with mean signal strength (optical density) in an age-adjusted analysis. Women negative for past and concurrent HPV 16 DNA had a significant trend of increasing optical densities associated with greater numbers of lifetime partners ($P_{Trend} < 0.001$). Conversely, the mean signal strength for those women who were ever HPV 16 DNA–positive during the study did not depend on lifetime numbers of sex partners ($P_{Trend} = 0.36$).**

Conclusions: **HPV 16 seropositivity is a surrogate for past HPV 16 infection. Circulating levels of antibodies to HPV 16 may reflect recent HPV 16 infection or the frequency of past HPV 16 infection.**

INFECTIONS WITH cancer-associated (oncogenic) human papillomaviruses (HPVs) are recognized as the primary cause of cervical cancer.[1–3] However, despite the essential role of HPV in carcinogenesis, relatively few HPV infections cause cervical cancer, precancerous lesions, cytologic high-grade squamous intraepithelial lesions, or underlying histologic cervical intraepithelial neoplasia (grade 2 or 3). For those investigating cervical cancer etiology, accurate assessment of past and present HPV infection has become critical to the next research step, including evaluation of events occurring subsequent to HPV infections (HPV cofactors) that may influence the outcome of an HPV infection.

One biomarker of HPV infection is HPV seropositivity.[4–6] Enzyme-linked immunosorbent assays that use HPV-like particles composed of the outer capsid protein L1 have emerged as the primary serological method of detection.[7] Previous studies have shown an association of HPV seropositivity with lifetime number of sex partners[6,8] and detection of genital HPV DNA (HPV DNA positivity),[4–6] indicating that HPV seropositivity most probably represents past infection with HPV.[9] However, there is less informa-

The authors thank Julie Buckland, Robert Banks, and Pei Chao in the data analysis group at Information Management Services, Inc., and Brenda Rush and Patti Lawler at Kaiser Permanente for their excellent technical support; and the physicians and nurse practitioners at Kaiser Permanente who expertly collected the specimens used in this research in the course of their clinical duties.

Supported by a series of contracts issued by the National Cancer Institute (NCI) to the collaborating clinical, coordinating, DNA testing, and data analysis groups. As the only exception, DNA testing of the first third of the specimens was provided by Cetus Corporation, Emeryville, CA (subsequently Roche Molecular Systems), under a formal Cooperative Research and Development Agreement with the NCI. R. K. was supported by grants from the Austrian National Bank (No. 7688) and the Austrian Science Foundation (FWF P13356-MED).

Correspondence: Philip Castle, PHD, MPH, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, MSC 7234, Bethesda, MD 20892. E-mail: pc95p@nih.gov

Received for publication June 6, 2001, revised July 25, 2001, and accepted August 1, 2001.

tion concerning the relationship of seropositivity to frequency of exposure (i.e., frequency of sexual intercourse), the timing of infection (i.e., time between DNA positivity and seropositivity), and the duration of infection (i.e., persistence of infection). Furthermore, there is little information about the determinants of the strength of seroreactivity. Herein we investigate these relationships in a subset of women who were previously tested for HPV seropositivity and for cervical HPV DNA.[5]

Materials and Methods

The study population was a subset of women evaluated in a nested case–control study of HPV 16–like particle seroreactivity and incident cervical neoplasia.[4,5,8] Blood specimens were taken at the time of cytologic diagnosis of the cases and at a matched time for controls. The results of HPV DNA tests were available both at diagnosis and earlier (from the time of enrollment of the entire cohort).[10,11] HPV serology and HPV DNA testing were performed with masking of case–control status. In this study, 447 women with valid serological results and with at least one valid HPV DNA test were included. Most had a valid cytologic assessment (n = 440), valid HPV DNA polymerase chain reaction testing at diagnosis (n = 426), and valid HPV DNA polymerase chain reaction testing at enrollment (n = 431). This subset included 18 women with incident high-grade squamous intraepithelial lesions and 72 with low-grade squamous intraepithelial lesions. The remaining subjects either had incident benign cytologic changes or were cytologically normal controls (approximately 3 controls were matched to each case by age, follow-up time, enrollment cytologic diagnosis, enrollment clinic, and participation status). Participants were observed for an average of 645 days (range, 278–1446 days); there was no difference in the average or range of follow-up time for each cytologic diagnosis.

A complete description of the pathology review has been published previously.[4,10,11] Potentially eligible subjects had all cervical smears and histopathologic slides, including pre-enrollment cervical smears, retrieved from the Kaiser Permanente archives for review. Smears were rescreened by a senior cytotechnologist. Final case definitions were determined by a pathology panel review of all smears and histopathologic specimens.

Serological testing was performed as previously described.[4,5,8] For this study, we restricted our analysis to HPV 16 seroreactivity because of the smaller number of women who were DNA-positive for the other HPV types. Seroreactivity to HPV 16 at the time of diagnosis was tested by enzyme-linked immunosorbent assays with use of HPV 16 L1–like particles.[5] Each serum sample was tested in quadruplicate and then averaged to determine the mean signal intensity (optical density [OD]) for each specimen. The mean coefficient of variability was 19%.[5] The cutpoint for HPV 16 seropositivity was set at 2.5 SD greater than the combined mean OD from a subset of (low-risk) women who were the least likely to have had a previous genital HPV infection (cutpoint OD = 0.235). ODs were adjusted between batches with use of a correction factor based on the mean OD of a reference serum tested in each batch.

Both at enrollment and at diagnosis, cervicovaginal lavage specimens were collected for HPV DNA testing. All specimens were tested by MY09/11 consensus primer polymerase chain reaction, combined with hybridization with use of type-specific oligonucleotide probes.[12,13] For clarity, detection of HPV 16 DNA at enrollment was defined as past HPV 16 DNA positivity (relative to diagnosis), whereas detection of HPV 16 DNA at diagnosis was defined as current HPV 16 DNA positivity.

Data were first analyzed by standard contingency table methods and stratified analyses to test the associations of sociodemographic, sexual, reproductive, smoking, and enrollment (e.g., past HPV DNA status) variables with current HPV 16 seroreactivity. To estimate relative risks, odds ratios and corresponding 95% CIs were calculated by means of unconditional logistic regression.

Analysis of variance was used to explore the relationships of covariates with the strength of seroreactivity, represented by the OD value, in the seropositive. Adjusted means were calculated with use of the lsmeans function of SAS software (SAS Institute, Cary, NC). Despite use of a Boxcox transformation for normalization,[14] OD values for HPV 16 seropositivity remained nonnormal, primarily because of deviations from linearity at the extremes on a Q-Q plot. Thus, nonparametric methods were used to confirm the validity of estimates from parametric methods. First, standard contingency tables were used to assess crude associations of covariates with the magnitude of seroreactivity, categorized according to quartile cuts. Those covariates found to be univariately associated with the magnitude were tested for significance by systematic addition of covariates into a regression model. The significant determinants were then added to an analysis of variance model; stratified analysis of a confounding variable was also used to isolate the effects of a covariate on the mean OD. Interactions between covariates were also tested for significance by means of analysis of variance. To test the statistical significance of dose–response (trend) relationships between covariates and seroreactivity, multilevel covariates were treated as continuous in a regression model and tested for whether the resultant β coefficient was nonzero. Finally, a nonparametric trend test[15] was performed on untransformed ODs for HPV 16 seropositivity.

Results

Increased lifetime and recent numbers (between enrollment and diagnosis) of sex partners, a history of genital

TABLE 1. Unconditional Logistic Regression Models for Human Papillomavirus Type 16 (HPV 16) Seropositivity

		Seropositivity (n = 447)					
		Univariate		Adjusted*		Adjusted†	
Variable	N	OR	95% CI	OR	95% CI	OR	95% CI
Lifetime male sex partners, no.							
1	158	1		1		1	
2	33	3.2	1.5–6.9	2.2	0.97–5.0	2.0	0.88–4.7
3–5	118	5.2	3.1–8.8	3.9	2.2–6.7	4.0	2.3–7.2
6–9	62	6.5	3.4–13	5.3	2.6–11	5.1	2.4–10.5
≥10	71	6.8	3.7–13	5.4	2.8–10	5.0	1.8–9.7
P_{Trend}		< 0.001		< 0.001		< 0.001	
Recent male sex partners,‡ no.							
0 or 1	353	1	NS		NS		
2	44	5.0	2.2–11				
≥3	44	2.2	1.1–4.2				
P_{Trend}		< 0.001					
History of genital warts							
No	404	1		NS		NS	
Yes	43	3.7	1.7–8				
Age (y)							
<20	59	1		NS		NS	
20–24	128	1.4	0.76–2.6				
25–34	144	1.1	0.57–1.9				
35–49	85	1.1	0.56–2.1				
≥50	30	0.48	0.19–1.2				
P_{Trend}		0.13					
Cytologic diagnosis							
Normal	350	1		1		1	
LSIL	72	2.5	1.4–4.2	1.4	0.76–2.6	1.4	0.76–2.7
HSIL	18	18	2.4–140	8.4	1.1–67	7.3	0.90–60
P_{Trend}		< 0.001		0.03		0.05	
Past HPV 16 DNA status							
Negative	404	1		1		1	
Positive	27	13	3.0–54	6.9	1.5–31	14	1.8–120
Current HPV 16 DNA status							
Negative	386	1				1	
Positive	40	4.0	1.8–8.8			0.90	0.35–2.3
Time (d) to diagnosis§							
≤480	148	1		NS		NS	
481–740	150	0.70	0.44–1.1				
≥740	149	0.77	0.49–1.2				
P_{Trend}		0.27					

*Adjusted for lifetime number of sex partners, past HPV 16 DNA positivity, and cytologic diagnosis.
†Adjusted for current HPV 16 DNA positivity, in addition to lifetime number of sex partners, past HPV 16 DNA positivity, and cytologic diagnosis.
‡No. of male sex partners between the time of enrollment and diagnosis.
§Time between enrollment and diagnosis.
HSIL and LSIL = high- and low-grade squamous intraepithelial lesions; NS = not significant when added to the multivariate model; OR = odds ratio.

warts, more severe cytologic diagnoses, and past and current HPV 16 DNA positivity (at baseline and at diagnosis) were associated with increased likelihood of current HPV 16 seropositivity (Table 1). Sociodemographic covariates (age and income), reproductive covariates (pregnancy and age at first menstrual cycle), other sexual behavior covariates (recent frequency of sexual intercourse and age at first intercourse), smoking, and time between enrollment and diagnosis were not associated with seropositivity. In a multivariate model, only lifetime number of sex partners ($P_{Trend} < 0.001$), severity of cytologic diagnosis ($P_{Trend} = 0.03$), and past HPV 16 DNA positivity (odds ratio = 6.9; 95% CI = 1.5–31) remained associated with seropositivity. The univariate association between number of recent sex partners and seropositivity was explained by the strong correlation between the number of recent sex partners and the number of lifetime sex partners ($P < 0.001$). In a model that included both past HPV DNA positivity and current HPV DNA positivity, the association of seropositivity and past DNA positivity was strengthened, whereas the association of seropositivity and current DNA positivity (odds ratio = 0.90; 95% CI = 0.35–2.3) was null (Table 1).

Increased lifetime and recent number of sex partners, age, more severe cytologic diagnoses, and past and current HPV

TABLE 2. Unadjusted and Adjusted Means of Signal Strength (OD) for Human Papillomavirus Type 16 (HPV 16) Seroreactivity Among Seropositive Women

	Signal Strength (OD) (n = 213)					
	Unadjusted		Adjusted*		Adjusted†	
Variable	Mean	*P*	Mean	*P*	Mean	*P*
Overall mean	0.303					
Lifetime male sex partners, no.						
1	0.089		0.147		0.172	
2	0.241		0.253		0.270	
3–5	0.346		0.412		0.415	
6–9	0.409		0.493		0.549	
≥10	0.451	< 0.001	0.526	< 0.001	0.558	< 0.001
P_{Trend}	< 0.001		< 0.001		< 0.001	
Recent male sex partners,‡ no.						
0 or 1	0.274		NS		NS	
2	0.314					
≥3	0.484	0.03				
P_{Trend}	0.009					
History of genital warts						
No	0.293		NS		NS	
Yes	0.368	0.26				
Age (y)						
<20	0.293		NS		NS	
20–24	0.408					
25–34	0.284					
35–49	0.253					
≥50	0.107	0.01				
P_{Trend}	0.01					
Cytologic diagnosis						
Normal	0.269		NS		NS	
LSIL	0.374					
HSIL	0.439	0.06				
P_{Trend}	0.02					
Past HPV 16 DNA status						
Negative	0.275		0.246		0.279	
Positive	0.615	< 0.001	0.465	0.003	0.482	0.03
Current HPV 16 DNA status						
Negative	0.259				0.314	
Positive	0.597	< 0.001			0.435	0.13
Time (d) to diagnosis§						
≤480	0.297		NS		NS	
481–740	0.299					
≥740	0.314	0.95				
P_{Trend}	0.76					

*Adjusted for lifetime number of sex partners, past HPV 16 DNA positivity, and age.
†Adjusted for current HPV 16 DNA positivity, in addition to lifetime number of sex partners, past HPV 16 DNA positivity, and age.
‡No. of male sex partners between the time of enrollment and diagnosis.
§Time between enrollment and diagnosis.
HSIL and LSIL = high- and low-grade squamous intraepithelial lesions; NS = not significant when added to the multivariate model.

16 DNA positivity were associated with increased mean signal strength (OD) in univariate analyses (Table 2). Other covariates were not associated with OD. In an age-adjusted multivariate model, only lifetime number of sex partners ($P_{Trend} < 0.001$) and past HPV 16 DNA positivity ($P = 0.003$) remained associated with mean signal strength. The association of current HPV 16 DNA positivity and seropositivity was nonsignificant in the fully adjusted model ($P = 0.13$).

Among those who were past and current HPV 16 DNA–negative, greater numbers of lifetime sex partners was associated with not only an increased odds ratio of HPV 16 seropositivity ($P_{Trend} < 0.001$) but also an increased mean signal strength ($P_{Trend} < 0.001$; data not shown). Conversely, among those who were past or current (ever) HPV 16 DNA–positive, greater numbers of lifetime sex partners were not associated with an increased odds ratio of HPV 16 seropositivity ($P_{Trend} = 0.91$) or with an increased mean signal strength ($P_{Trend} = 0.36$; data not shown). When a nonparametric trend test was used on untransformed ODs, the trend of greater numbers of lifetime partners and mean ODs was significant among the HPV DNA–negative

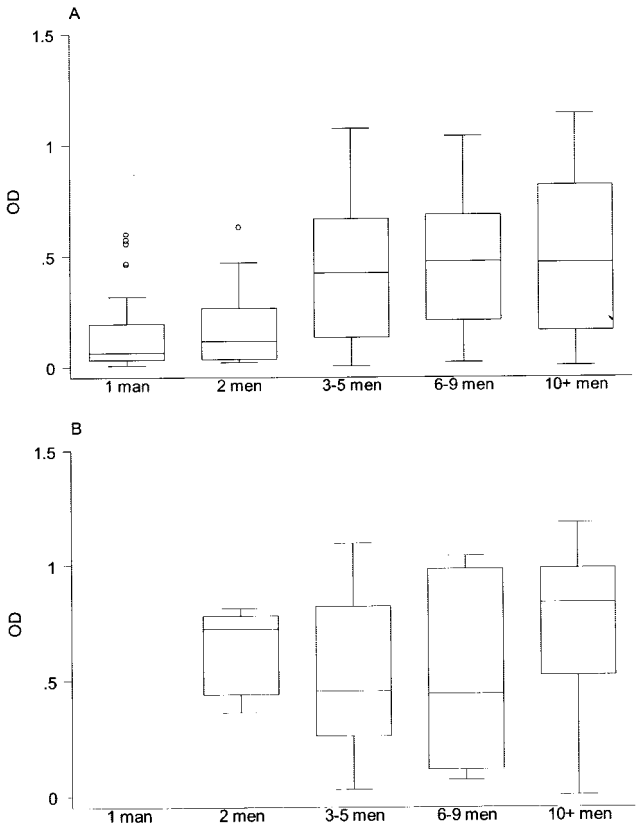

Fig. 1. Box-and-whiskers plot of untransformed optical density (OD) for human papillomavirus type 16 (HPV 16) seroreactivity among seropositive women, versus lifetime number of sex partners, stratified by HPV 16 DNA status (at enrollment or at diagnosis). The nonparametric trend test[13] among HPV 16 DNA–negative women (A) was significant ($P < 0.001$), and among HPV 16 DNA–positive women (B) it was nonsignificant ($P = 0.059$).

women ($P_{\text{Trend}} < 0.001$) and nonsignificant among those ever positive for HPV 16 DNA ($P_{\text{Trend}} = 0.06$; Figure 1). Although the overall interaction term between number of lifetime partners and past HPV 16 DNA positivity was not statistically significant, the individual interaction terms for 3 to 5 men ($P = 0.04$) and 6 to 9 men ($P = 0.04$) were statistically significant.

Discussion

HPV 16 seropositivity appears to be linked primarily with past exposure to or infection with HPV 16, as measured directly by past HPV 16 DNA positivity or indirectly by number of lifetime partners. Note that when added into a multivariate model, current HPV 16 DNA was not associated with seropositivity, whereas the association of past DNA positivity was apparently strengthened. This finding suggests that distant infections are more closely linked to current seropositivity and is consistent with the strong association of seroconversion and high-grade squamous intraepithelial lesions, which in this cohort[11] and another cohort[16] tended to be diagnosed an average of approximately 4 years after the initial HPV infection. In addition, lifetime number of partners appeared to explain the univariate association of recent partners with seropositivity.

The trend of increasing (adjusted) mean signal strength, as measured by OD, with increasing number of lifetime partners may suggest that women with more sex partners in their lifetimes have higher titers of HPV antibodies than do women with fewer partners. The increased signal strength in women with greater numbers of sex partners was also observed for HPV 18, 31, and 45 seropositivity, albeit less robustly than for HPV 16 (data not shown). This finding persisted in the absence of detectable HPV 16 DNA, which was also shown to be a determinant of OD. Furthermore, this association could not be explained by increased positivity for all serotypes as the result of increased number of lifetime partners; a recent report suggests there is little or no cross-reactivity between serotypes when tested with L1 virus-like particles.[17]

There was no link between recent frequency of sexual intercourse and mean OD. Furthermore, there were some near-significant differences in the mean OD by cytologic diagnosis for HPV 16 seropositivity but not for HPV 18, 31, and 45 seropositivity (data not shown). These findings hint at an unresolved relationship among enhanced humoral response due to repeated infection, the timing of the infection, the HPV type, the level of immunity at the time of infection, and whether an infection needs to be established to constitute a sufficiently large viral load to trigger an immune response. Prospective studies may be able to address these questions.

There are limitations to this study. First, although the overall size of the study was not small, the subgroups were. Second, we lacked questionnaire data on male partners' sexual behavior. Since men are vectors for transmission, their sexual behavior will impact on the timing and dose of their female partners' exposures to HPV. The natural history of HPV infection in men, i.e., when the viral load is the greatest, when they are most contagious, and how long the infection is maintained, is not well understood. Third, we did not have a serum specimen from the time of enrollment. Seropositivity at enrollment would have permitted a comparison of past seroreactivity with present seroreactivity and number of recent partners. Fourth, we have no data to correlate OD values with specific antibody concentrations. Finally, immunologic data on cervical secretions were not collected. The local immunity of the cervix is part of a separate immune network (common mucosal immune system) that is at least somewhat independent of the systemic immune system.[18] Thus, immune response to HPV at the cervix may differ from the systemic response, and the threshold viral dose for a local and systemic immune boost may differ. We have begun to investigate determinants of

local immunity,[19] and future studies will evaluate the relationship of local immunity and sexual behavior.

Despite the limitations of this study, the evidence presented is consistent with the view that DNA positivity indicates current infection and seropositivity indicates past and ongoing infection.[9] In a recent study assessing seroconversion of incident HPV infections, HPV 16 and HPV 18 seroconversions typically occurred between 6 and 12 months after DNA positivity, whereas HPV 6 (a nononcogenic type) seroconversion was more likely to be concurrent with DNA positivity.[20]

In cross-sectional studies of cervical cancer, it may still prove useful to examine seropositive (for an oncogenic type), HPV DNA–negative, cytologically normal women as controls for assessment of HPV cofactors. These women were at risk of disease because they were infected by HPV in the past (indirectly assessed by seropositive status), but the infection was cleared or suppressed before development of high-grade squamous intraepithelial lesions. However, it was recently observed that only a fraction of women with high-grade lesions were HPV-seropositive,[21] suggesting that the current serologic methods are still insensitive for detection of all HPV-associated disease.

The association of greater signal intensity with greater lifetime number of male sex partners, if real, may reflect recent HPV 16 infections and frequency of past HPV 16 infections. Indeed, women with more lifetime sex partners were more likely to have greater numbers of recent partners and thus are probably more likely to have been recently infected with HPV 16 at any point in time because of the greater frequency of infection. Greater signal intensity may be a surrogate or index of lifetime number of infections/exposures. This finding may have important implications with regard to antibody titers in women participating in HPV 16 vaccine trials; subsequent HPV 16 infections could theoretically increase and prolong immunity from vaccination. However, an alternative interpretation is that, despite the presence of antibodies to HPV as the result of multiple infections, women can still acquire an HPV infection that subsequently causes an additional boost to antibody titers. It is noteworthy that serum titers of antibody to HPV 16 in women participating in trials of a vaccine with HPV 16 virus-like particles are significantly greater than titers resulting from natural infection.[22] It remains to be determined whether antibodies to HPV in cervical secretions at any concentration are prophylactic against the sexual transmission of HPV.

References

1. Bosch FX, Manos MM, Munoz N, et al. Prevalence of HPV DNA in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995; 87:796–802.
2. Walboomers J, Jacob M, Manos M, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 198:12–19.
3. Munoz N. Human papillomavirus and cancer: the epidemiological evidence. J Clin Virol 2000; 19:1–5.
4. Wideroff L, Schiffman MH, Nonnenmacher B, et al. Evaluation of seroreactivity to human papillomavirus type 16 virus–like particles in an incident case-control study of cervical neoplasia. J Infect Dis 1995; 172:1425–1430.
5. Wideroff L, Schiffman M, Haderer P, et al. Seroreactivity to human papillomavirus types 16, 18, 31, and 45 virus–like particles in a case-control study of cervical squamous intraepithelial lesions. J Infect Dis 1999; 180:1424–1428.
6. Sun Y, Eluf-Neto J, Bosch FX, et al. Serum antibodies to human papillomavirus 16 proteins in women from Brazil with invasive cervical carcinoma. Cancer Epidemiol Biomarkers Prev 1999; 8:935–940.
7. Kirnbauer R, Hubbert NL, Wheeler CM, Becker TM, Lowy DR, Schiller JT. A virus-like particle enzyme-linked immunosorbent assay detects serum antibodies in a majority of women infected with human papillomavirus type 16. J Natl Cancer Inst 1994; 86:494–499.
8. Wideroff L, Schiffman MH, Hoover R, et al. Epidemiologic determinants of seroreactivity to human papillomavirus (HPV) type 16 virus–like particles in cervical HPV-16 DNA–positive and –negative women. J Infect Dis 1996; 174:937–943.
9. Olsen AO, Dillner J, Gjoen K, Magnus P. Seropositivity against HPV 16 capsids: a better marker of past sexual behaviour than presence of HPV DNA. Genitourin Med 1997; 73:131–135.
10. Schiffman MH, Bauer HM, Hoover RN, et al. Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. J Natl Cancer Inst 1993; 85:958–964.
11. Liaw KL, Glass AG, Manos MM, et al. Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. J Natl Cancer Inst 1999; 91(11):954–960.
12. Bauer HM, Greer CE, Manos MM. Determination of genital human papillomavirus infection by consensus PCR amplification. In: Herringon CS, McGee JO'D, eds. Diagnostic Molecular Pathology: A Practical Approach. Oxford: Oxford University Press, 1992:131–152.
13. Hildesheim A, Schiffman MH, Gravitt PE, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. J Infect Dis 1994; 169:235–240.
14. Box GEP, Cox DR. An analysis of transformations. J Royal Stat Soc (Series B) 1964; 26:211–243.
15. Cuzick JA. Wilcoxon-type test for trend. Stat Med 1985; 4:87–90.
16. Herrero R, Hildesheim A, Bratti C, et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst 2000; 92:464–474.
17. Giroglou T, Sapp M, Lane C, et al. Immunological analyses of human papillomavirus capsids. Vaccine 2001; 19:1783–1793.
18. Mestecky J, Fultz PN. Mucosal immune system of the human genital tract. J Infect Dis 1999; 179:S470–S474.
19. Hildesheim A, McShane LM, Schiffman M, et al. Cytokine and immunoglobulin concentrations in cervical secretions: reproducibility of the Weck-cel collection instrument and correlates of immune measures. J Immunol Methods 1999; 225:131–143.
20. Carter JJ, Koutsky LA, Hughes JP, et al. Comparison of human papillomavirus types 16, 18, and 6 capsid antibody responses following incident infection. J Infect Dis 2000; 181:1911–1919.
21. Anttila T, Saikku P, Koskela P, et al. Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA 2001; 285:47–51.
22. Harro CD, Pang YY, Roden RB, et al. Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus–like particle vaccine. J Natl Cancer Inst 2001; 93:284–292.